[Chem. Pharm. Bull.
22(12)2803—2811(1974)]

*UDC 615.011.3.014*

# Mixing of Pharmaceutical Powders examined by Use of Colored Particles: Discussions on Coloration Difference

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(Received March 11, 1974)

A new mixing index based on Hunter's color difference formula was applied to the three-component mixing of antipyrin, lactose and lactose colored with pigment (tracer powder of antipyrin). The mixing was conducted in a twin-shell mixer. The scale of scrutiny of this index was comparable with that of the mixing index used for the active ingredient. The concept of the uneven coloration is useful to follow the progress of mixing and to confirm the complete mixing. The relationship between the behavior of the active ingredient and tracer powder in the earlier stages of mixing was examined by the separate determination of antipyrin and pigment.

It has been the custom in judging the state of mixing of white powders to determine directly the objective ingredient by chemical or physical means to obtain some kind of a mixing index, or to mix colored particles together with it and to indirectly estimate the state of mixing of the objective ingredient from visual observation of uneven coloring. The latter method is advantageous in being able to shorten the time and lessen the labor of analysis but there would naturally be individual difference because of subjective factors of having to rely on the visual observation of an individual, however much the observation conditions are made uniform. It is also difficult to set a definite standard for uneven coloration. In the field of pharmacy, color is used to a great extent as a supplementary means for the discrimination of products and for quality control during manufacture of pharmaceutics. For example, as indicated in the "Pharmacy Manual,[2]" pigments are used in mixing a minute quantity of powerful drugs with a diluent to a relatively high dilution, *e.g.*, Reserpine Powder 1000 Trituration and Codeine Phosphate Powder 100 Trituration. In such a case, color is used to indicate that the mixed powder is a trituration of powerful drug and also as an index to confirm the performance of the complete mixing of powdered ingredients by visual inspection. However, there is still an ambiguity as to the correlation between disappearance of uneven coloration and uniform mixing of the active ingredient and, as far as the authors are aware, studies on such a point have never been published.

There has been a few reports[3-5] on the determination of the degree of mixing of solids by optical methods. In such a method, dispersion of observed values due to non-uniformity of conditions in inserting the photocell probe into the powder mixture was the problem, in addition to the necessity of having to prepare a calibration curve of a complete mixture for each of the mixing systems in order to correlate the concentration of the objective component with optical values.

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1) Location: *a) Motoyama-Kitamachi, Higashinada-ku, Kobe; b) Yoshida-Shimoadachi-cho, Sakyo-ku, Kyoto.*

2) Japan Pharmaceutical Association (ed.), "Pharmacy Manual," Yakuji-Nippoh-sha, Tokyo, 1972, p. 27.

3) J.B. Gray, *Chem. Eng. Progr.*, 53, 25J (1957).

4) M.D. Ashton, C. Shofield, and F.H.H. Valentin, *Chem. Eng. Sci.*, 21, 843 (1966).

5) T. Yano, M. Satoh, and Y. Mineshita, *J. Res. Assoc. Powder Tech., Japan*, 9, 244 (1972).

Having noted the presence of a direct correlation between a colorimetric method and human color perception, the authors attempted the application of color and color-difference meter for measurement of the degree of mixing in order to introduce objectivity into the above-mentioned visual inspection method. It was thereby found that numerical expression of uneven coloration would give a mixing index that can be used as a standard for color difference and suitability of the existing visual inspection method was proved. It was also found that particle distribution of the active ingredient in the initial period of mixing can be estimated visually by this method which offered an important index for a mixing procedure.

## Experimental

**Materials**——The active ingredient used was antipyrin Japanese pharmacopeia (JP) and the diluent was spray-dried lactose. These samples were each sifted to the desired granularity. The colored particles used as the tracer were prepared by gradually adding 100 ml of 2% (w/v) EtOH solution of Rose Bengal (Merck product) to 400 g of the spray-dried lactose placed in a mortar, mixing thoroughly, air dried at room temperature, and passed through a 100 mesh sieve. Special care was taken for this air drying in order to prevent pigment migration. Powder characteristics of these samples are shown in Table I. It will be seen from this Table that flowability of pigment-coated lactose has remarkably increased compared to that of pure lactose due to the lubricating effect of the pigment.

Table I. Characteristics of Sample Powders Used

| Materials | Antipyrin | Tracer | S.D. Lactose |
|---|---|---|---|
| Mesh size | 65/100 | −100 | −100 |
| Mean particle size (μ)$^{a)}$ | 84 | 21 | 19 |
| Specific surface Area (cm$^2$/g)$^{b)}$ | 647 | 1826 | 1997 |
| Density (g/cm$^3$)$^{c)}$ | 1.251 | 1.528 | 1.526 |
| Bulk density (g/cm$^3$)$^{d)}$ | 0.600 | 0.546 | 0.419 |
| Repose angle (degree)$^{e)}$ | 48.5 | 49.6 | 58.8 |

*a*, *b*) measured by air permeability method
*c*) measured by Beckmann air comparison pycnometer
*d*) measured according to JIS Z 2504—1966
*e*) table diameter=8 cm, dropping height=9 cm

**Mixer and Sampling**——The twin-shell mixer of 2.54-liter capacity (Tokuju Kosakusho, Tokyo), with cyrinders placed at 90° angle to each other, was used. Revolution ($N$) during the experiment was 25 rpm. For the charging, 1 g of antipyrin was placed first, 5 g of the colored particles was placed over it uniformly, and 494 g of lactose was finally added. After a definite period of mixing, the mixture was transferred to a square box of 20 cm sides, and 20 spot samples of *ca.* 500 mg each were collected from this mixture, using a table of random numbers. Color difference analysis and determination of the pigment and antipyrin were carried out with these samples. Samples for mixing were renewed for each mixing procedure.

**Color Difference Analysis and Quantitative Determination**——For color difference analysis, the collected sample was placed in a cyrindrical quartz cell of 10 mm in inner diameter, close packing of the sample was assured by tapping, and surface color of the sample in the *Lab* system,[6] was measured with the color and color difference meter, Model ND-K6B (Nippon Denshoku Kogyo, Tokyo). Error due to variation in the state of sample filling was found to be negligibly small compared to uneven coloration, and there was entirely no bias in measured values due to direction of the sample because this meter uses the integral globe system. Measured values of $L$, $a$, and $b$ of the colored particles showed less than 0.14% in coefficient of variation, and the sample can be regarded as sufficiently uniform in color.

After the color measurement, each sample was diluted suitably with distilled water to dissolve lactose, pigment and antipyrin, and the quantity of Rose Bengal and antipyrin in the solution was determined by the two-absorbance reading method with a spectrophotometer, with maximnm absorbance of Rose Bengal at 548 nm and antipyrin at 242 nm. In this case, effect of the pigment on the absorbanc of antipyrin could be neglected after more than 200 times of mixing.

**Mixing Degree for Active Ingredient and Tolerance Level**——According to the method of Okada, Matsuda and others,[7] the degree of mixing of the active ingredient was indicated by the coefficient of variation of

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6) JIS Z 8730$^{-1970}$: Methods for Specification of Color Differences for Opaque Materials.
7) J. Okada, Y. Matsuda, S. Morita, Y. Wada, and H. Ohnishi, *Yakugaku Zasshi*, **88**, 827 (1968).

variance of concentration, using the mean concentration calculated from the quantity of the charge. Tolerance level was 2.5% by the coefficient of variation for over 95% of the population to be within the stipulated range of mean concentration, $C_0$, of $(1-0.05)C_0 \leq S \leq (1+0.05)C_0$.

## Result and Discussion

### Colorimetric Measurements

Physical evaluation of color has hitherto been made by a spectrophotometric method but colorimetry must be used to carry out quantitative measurement of color related to human visual perception. According to Grassman's law, color is determined by three independent attributes and, therefore, a color can be indicated as a point in three-dimensional space. In the present report, color is expressed by the *Lab* system because this system has approximately uniform step related to human color perception and the following relation is established between the values of $L$, $a$, and $b$, and the tristimulus values,8) $X$, $Y$, and $Z$.

$$L = 10Y^{1/2} \tag{1.1}$$

$$a = 17.5(1.02X - Y)/Y^{1/2} \tag{1.2}$$

$$b = 7.0(Y - 0.847Z)/Y^{1/2} \tag{1.3}$$

where, $L$: lightness in the *Lab* system
$a,b$: chromaticity coordinates in the *Lab* system
$X,\ Y,\ Z$: tristimulus values in the *XYZ* system

In this system, colorimetric measurement of a complete mixture of lactose and colored particles in various concentrations gives the result shown in Fig. 1.

This graph indicates that the lactose is a non-colored powder (saturation$=\sqrt{a^2+b^2}=0$)' and that the saturation (chroma) of the mixed powders rises with increasing addition of the colored powder. The value of $a$ changes the most among $L$, $a$, and $b$, and a change in the point $(a, b)$ on the $a$—$b$ plane indicates that the color of the mixed powder rapidly approaches reddish violet with the addition of the colored powder. If the color changes of these mixtures were to follow Grassman's law perfectly, equations (2.1) to (2.3) should establish for each value of $L$, $a$, and $b$, and the curves of the measured values in Fig. 1 should agree with three lines connecting the points of 0% and 100% of lactose and the colored powder. However, the measured curves deviate greatly from these theoretical lines (2.1 to 2.3).

$$L_x = (100-x)L_{\mathrm{L}} + xL_{\mathrm{T}}/100 \tag{2.1}$$

$$a_x = (100-x)a_{\mathrm{L}} + xa_{\mathrm{T}}/100 \tag{2.2}$$

$$b_x = (100-x)b_{\mathrm{L}} + xb_{\mathrm{T}}/100 \tag{2.3}$$

where, $x$: concentration of colored particles (wt%)
subscript $L$: lactose
$T$: tracer (colored particle)

The Lambert-Beer's law for transmitted light was applied to the $L$ value which is related to the lightness of a mixed powder (dotted line in Fig. 1), and the measured curve did not agree with it, similar to the result obtained by Yano and others5) who applied it for the reflectance of a mixture of glass beads. Therefore, numerical differentiation of each curve in Fig. 1 was made by the five-point method and its result is shown in Fig. 2. It will be seen from this graph that the rate of change of $L$, $a$, and $b$ against the amount added ($dL/dx$, $da/dx$, and $db/dx$) changes rapidly at a very low concentration of the additive, and the rate of change of $a$ is especially marked. This change gradually decreases up to around 20% addition and there is hardly any change above this concentration. It became clear from Fig. 1 and 2 that the

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8) A.C. Hardy, "Handbook of Colorimetry", Technology Press of MIT, Cambridge, Mass., 1936, pp. 32—48.

color of a mixed powder deviates markedly from the tendency predicted theoretically in the region of a small addition of the colored substance. Such a complicated behavior of a powder to reflected light suggests that luster and surface characteristics of a powder, which are excluded from Grassman's law, have a great effect on the result of colorimetric measurement. Goodhart and others[9] obtained the same result as the present one with respect to saturation and lightness of color in another color system.

### Expression of Color Differences and Mixing Index of Color Difference Basis

Color difference, $\Delta E$, in the color of materials is generally expressed by the distance between two points in color space. According to Japanese Industrial Standard JIS Z 8730,[6] Hunter's color difference formula is expressed by the following equation (3).

$$\Delta E(Lab) = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2} \tag{3}$$

where, $\Delta E(Lab)$: color difference in the $Lab$ system

$\Delta L$, $\Delta a$, $\Delta b$: difference between two lightnesses, $L$, and those between chromaticity coordinates, $a$ and $b$ in the $Lab$ system

Consequently, by using equation (3), mean color difference (uneven coloration) $M$ of $n$ number of samples from these complete mixture will be expressed by the following equation:

$$M = \overline{\Delta E} = 1/n \sum_{i=1}^{n} \{(L_i - L_c)^2 + (a_i - a_c)^2 + (b_i - b_c)^2\}^{1/2} \tag{4}$$

where, subscript $c$: complete mixture

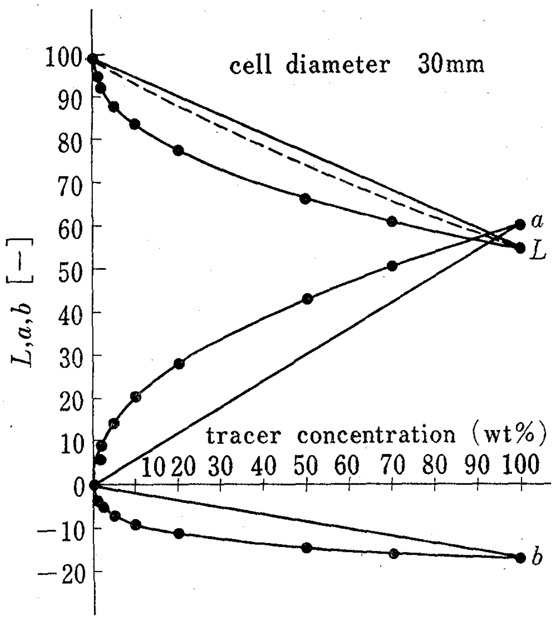

Fig. 1. Colorimetric Data of Complete Mixtures between Lactose and Tracer Particles

—●—: experimental curves
——: theoretical lines based on Eqn. (2.1)—(2.3)
------: imaginary curve based on Lambert-Beer's Law

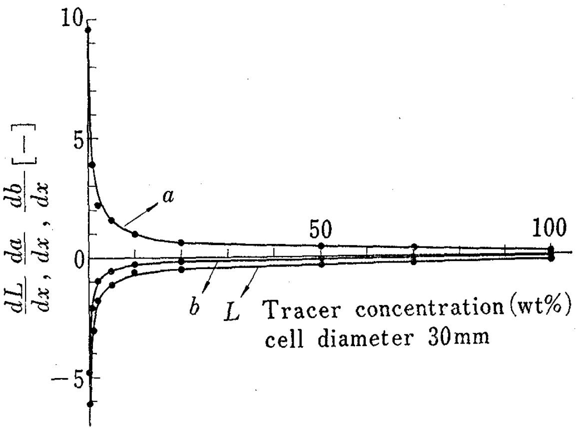

Fig. 2. Rates of Change of $L$, $a$, and $b$ against Tracer Concentration in Fig. 1

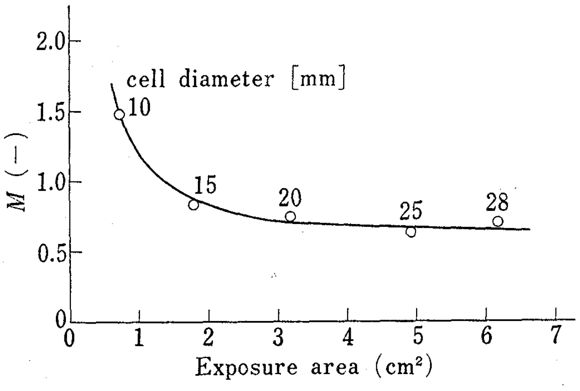

Fig. 3. Effect of Exposure Area on Mean Color Difference, $M$

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[9] F.W. Goodhart, M.A. Kelly, and H.A. Lieberman, *J. Pharm. Sci.*, **54**, 1799 (1965).

As will be clear from the foregoing statement, the values of $L_c$, $a_c$, and $b_c$ of the complete mixture do not agree with theoretical curves (2.1 to 2.3) even if the additive concentration and colorimetric values of lactose and colored particles are given. In the present case, precision of the calibration curve was unstable in the region of small addition (1% w/w), and above values could not be calculated as was done in the case of the active ingredient. Consequently, colorimetric values of the complete mixture (mixed for 80 min) was taken as $L_c$, $a_c$, and $b_c$ values.

According to Kawakami,[10] color difference of adjacent materials should be below 1 in $\Delta E$ when strict equality of color is required, and he stated that this degree of difference is difficult to discriminate by human visual perception.[11] For this reason, the mean color difference in equation (4) cannot completely express unevenness of color by visual perception. Therefore, the authors propose the following new mixing index based on color difference, in order that the mean value of the population, taking the 20 samples as representing the powder population, will be below the above-mentioned tolerance level ($\Delta E_S=1$) in color difference from the complete mixture at the 95% probability.

$$M_c = \overline{\Delta E} + 1.645\sigma \leq \Delta E_s = 1 \tag{5}$$

where, $\sigma^2=1/20\sum_{i=1}^{20}(\Delta E_i-\overline{\Delta E})^2$

In order to examine the effect of the exposed surface of the cell on unevenness of color, examinations were made on the relationship between $M$ calculated from equation (4) and exposed surface area of the cell, using 30 samples obtained from an incomplete mixture (cumulative revolution, 30 times), and its result is shown in Fig. 3. The exposed surface area was changed consecutively without changing the kind of the sample filled in the cell and $\Delta E$ was calculated at each change. The value of $\overline{\Delta E}$ for each of the same exposed surface area was calculated for 30 samples. It will be clear from Fig. 3 that the mean color difference, $M$, increases with decrease in cell area and this is especially marked when the cell diameter is 10 mm. Since the cell area corresponds to the visual field in the case of human visual judgement, the result shown in Fig. 3 sufficiently explains the general tendency that the judgement

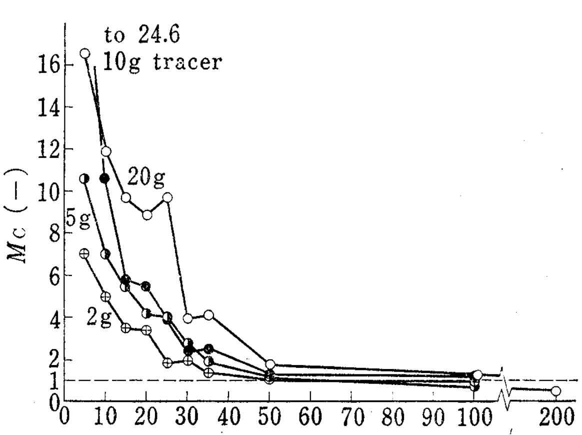

Fig. 4. Effect of Tracer Concentration on the Development of Color

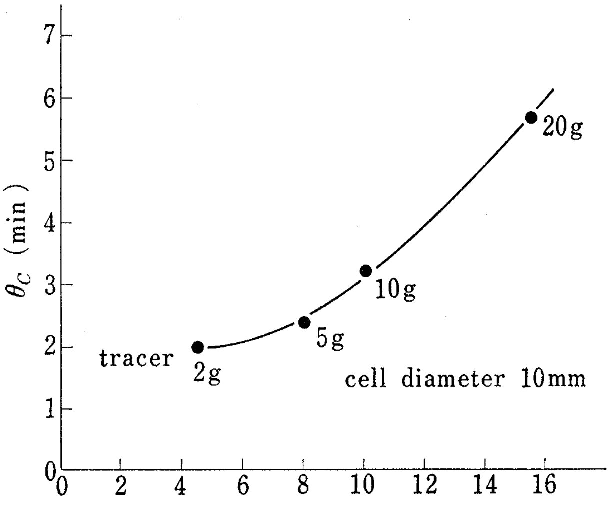

Fig. 5. Relation between Required Mixing Time, $\theta_c$ and Color Differences between Complete Mixture and Lactose

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[10] S. Yamaguchi and G. Kawakami, "Shi kisai Kogaku Nyumon," Ohmu-sha, Tokyo, 1967, p. 69.

[11] G. Kawakami, *Private Communication.*

standards become stricter as the visual field becomes narrower. For this reason, subsequent colorimetric measurements were made with a cell of 10 mm in diameter.

## Development of Color

When a colored powder or a pigment is used as a tracer in mixing operation, the concentration of the additive and the color characteristics of the tracer, besides its physical properties and color difference between the tracer and a diluent must affect development of color. Fig. 4 shows the periodical change in color development during the mixing operation by the addition of colored powder in various concentrations. It will be seen from this graph that the rate of color development depends on the concentration of the additive and the lower the concentration, the faster the unevenness of color reaches the tolerance level. Data in Table I show that the colored particles have better flow properties than pure lactose. Consequently, assuming from the flow properties alone, the rate of the color development should be greater with higher concentration of the colored particles but the actual result was the reverse of that. This fact suggests that the color development is affected less by the physical properties of a powder like flowability and more by the color characteristics. The desired mixing time, $\theta_c$, obtained from the intercept of the curves for each of the concentrations and the tolerance level is plotted against the color difference between a complete mixture containing the colored powder and pure lactose in Fig. 5. It will be seen from this graph that the color difference between the complete mixture and pure lactose naturally becomes larger as the amount of the colored powder added increases, but the color difference between the complete mixture and the colored powder inversely becomes smaller and the period of mixing required becomes longer. Consequently, this color difference may be said to correspond to the driving force in the color development.

The foregoing is only on the behavior of the colored powder and lactose but the main objective of the present work was to examine the correlation, if any, between the colored particles and active ingredient particles in the state of mixing. As is evident from Table I, the density of the colored powder and lactose is the same but they differ in their flowability. Therefore, it can be considered that the mixing in this work is a three-component system which is composed from three kinds of powders having different particle characteristics. Fig. 6 shows the degree of mixing of the colored powder and the active ingredient in the mixing of this three-component system by the color difference basis and the concentration basis respec-

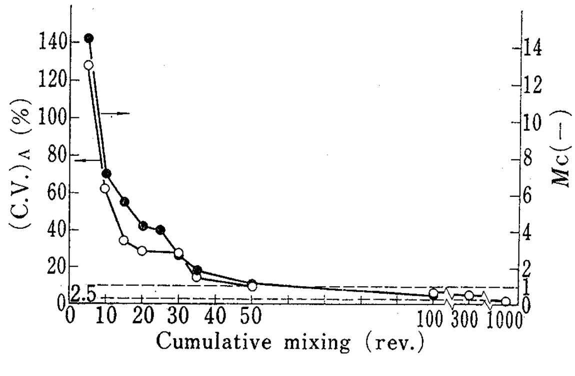

Fig. 6. Development of Color and Mixing of Active Ingredient

—●—: tracer (color difference basis)
—○—: antipyrin (concentration basis)

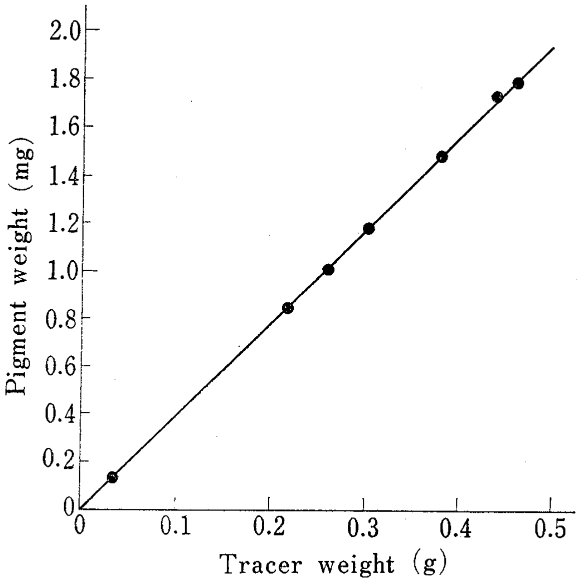

Fig. 7. Linearity between Tracer Weight and Pigment Weight Contained

tively. The period required to reach the tolerance level ($M_c=1$, coefficient of variation= 2.5%) was *ca.* 2 min for the colored powder but over 12 min (cumulative revolution, 300 times) for the active ingredient. At the dilution of this mixed system (1: 500), ±10% of the stipulated quantity is within the tolerance range according to the JP, and this will be *ca.* 9.3% when converted into the coefficient of variation. In this case, therefore, disappearance of uneven color can be regarded as the criterion for complete mixing of the active ingredient.

## Correlation between Behavior of Active Ingredient and Colored Powder

It was shown above that the end of color development signified complete mixing but, in order to reveal the relation between unevenness of color and distribution of active ingredient during the stages of mixing until this end point is reached, measured values up to 30 times of cumulative mixing in Fig. 6 were examined and its result is shown in Fig. 7. As shown in Fig. 7, as far as the colored powder was concerned, an extremely good quantitative relationship was found to exist between the weight of the pigment coated on the particles and the weight of colored particles, within the weight range of the increment size in the present experiment. Therefore, it became possible to calculate the weight levels of the colored powder from the determination of the pigment in a given sample. Concentration of the active ingredient and the colored powder in each sample obtained by this means is plotted against each of cumulative revolutions in Fig. 8, from which it will be seen that the two are in good correlation after five cumulative revolutions. The fact that this relationship is further improved with the progress of mixing is endorsed by the correlation coefficient shown in Fig. 9. Thus, the active ingredient and colored powder were found to show a closely related behavior during the initial stage of mixing, and these observations are considered to be very useful in designing

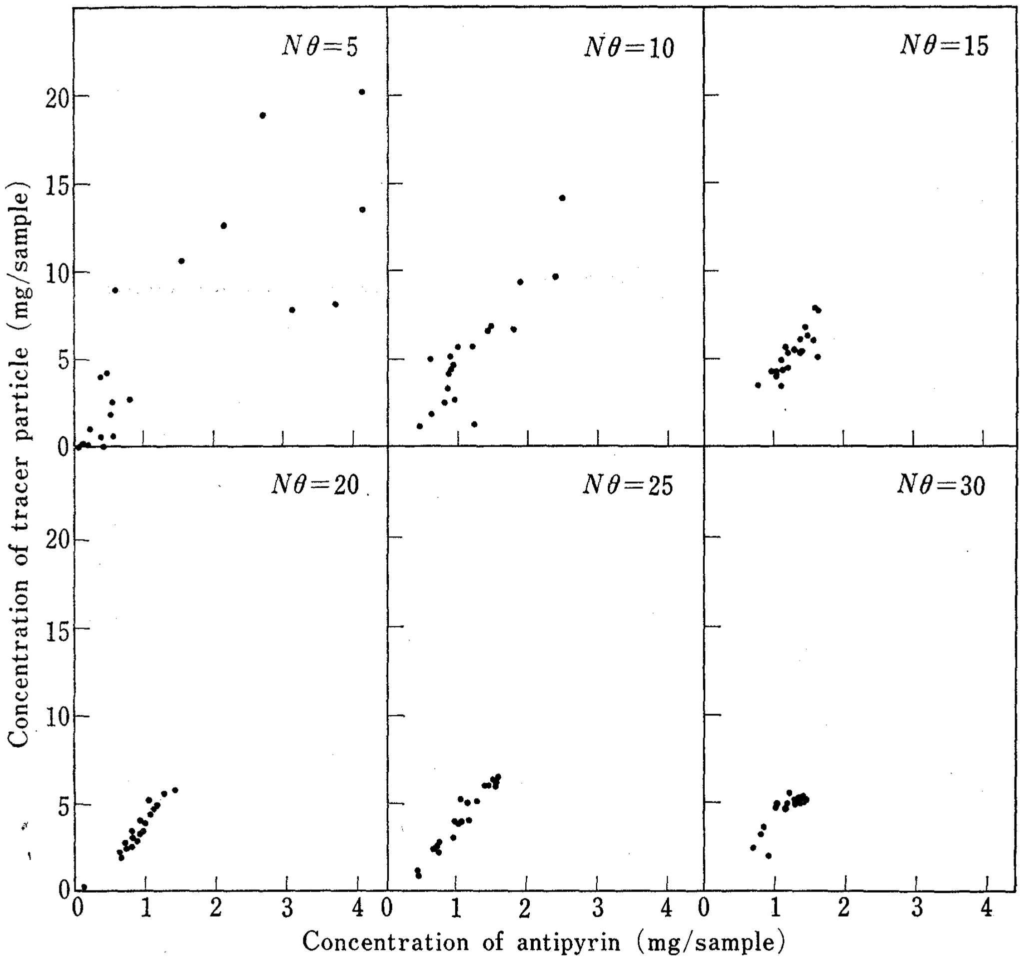

Fig. 8. Correlation between Concentrations of Antipyrin and Those of Tracer

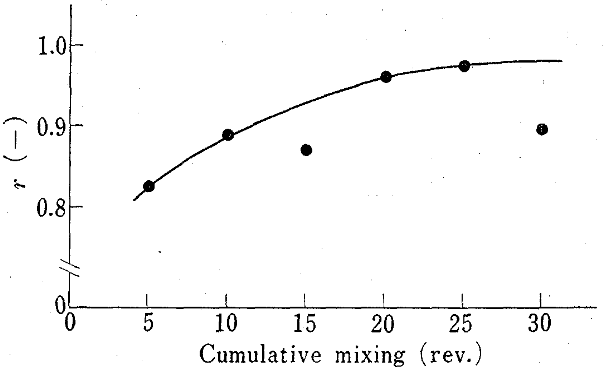

Fig. 9. Improvement of Correlation State between Concentrations of Antipyrin and Those of Tracer due to the Progress of Mixing

a new type of mixers and in establishing the optimal conditions for mixing operation. Based on these facts, the degree of mixing of the colored powder based on its concentration and the degree of mixing from color difference basis were compared and a good correlation was found to exist between these, as shown in Fig. 10. At the same time, as shown in Fig. 11, the degree of mixing of the colored powder corresponded well with that of the active ingredient. These facts indicate that measurement of unevenness of color during the mixing operation can predict indirectly the state of mixing of the active ingredient, and that the combination of the use of a new mixing index of color difference basis and colorimetric method is useful as a simple method for judging the degree of mixing of the active ingredient.

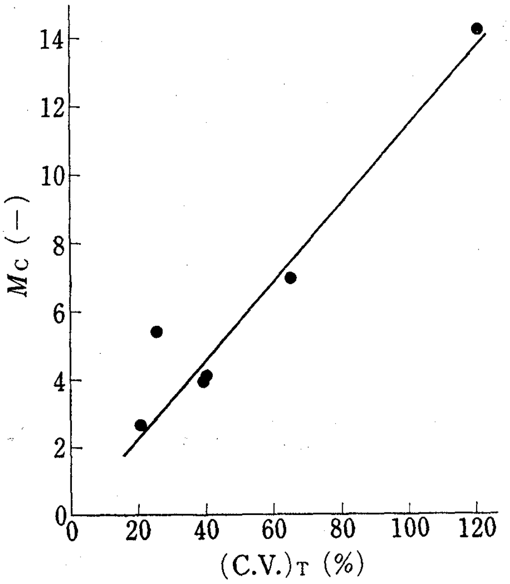

Fig. 10. Correlation between the Mixing Index based on Color Differences, $M_C$ and That based on Weight Concentration of Tracer, $(C.V.)_T$

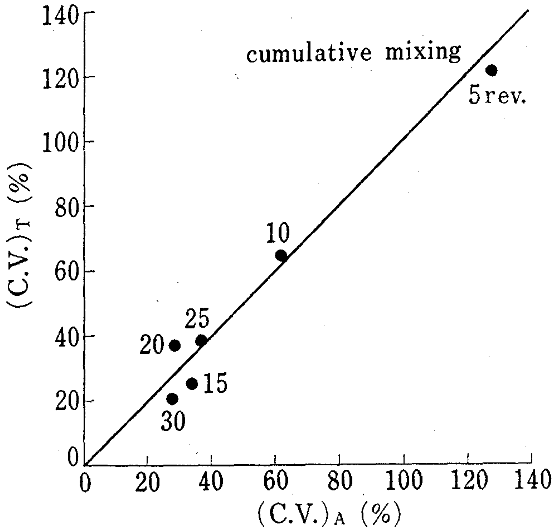

Fig. 11. Correlation between the Mixing States of Antipyrin and Those of Tracer

A: antipyrin T: tracer

Expression of unevenness of color by physical data of the degree of mixing from color difference, and examination of a relationship in the behavior between the active ingredient and colored powder have offered a valuable information for the method of visual judgement of mixing. However, there still remain numerous problems to be solved, such as the establishment of measurement conditions, functional tests, and statistical processing of data, before these results can be directly related to visual judgement.

## Conclusions

The theory of color difference was used to examine the use of color difference meter in judging the mixing of a three-component system of antipyrin, pure lactose, and lactose colored with Rose Bengal (tracer powder), and the following conclusions were drawn.

1. Addition of a tracer amount of the colored powder greatly changed the colorimetric values of a complete mixture of the colored powder and these deviated markedly from the theoretically predicted tendency.
2. A new mixing index of equation (5) was proposed for the color difference basis and used to evaluate the state of mixing of the mixture by a twin-shell mixer. This equation seems to be applicable for the judgement of unevenness of color of pharmaceutical products.
3. Rate of color development during the mixing operation was found to be faster when the concentration of colored powder added was low and the time required for unevenness of color to reach the tolerance level was short.
4. Close relationship of the state of mixing of the colored powder and particles of active ingredient (antipyrin) was proved from the degree of mixing by color difference basis and concentration basis. It was found that the disappearance of uneven color can be taken to estimate the sufficient mixing of the active ingredient.